

Bacterial Transformation

Plasmid DNA are circular molecules of double-stranded DNA that can replicate independently of the bacterial chromosome. Plasmids typically contain genes that encode proteins advantageous to the host cell. For example, bacterial resistance to antibiotics or toxins in the environment may be conferred by the plasmid DNA.

In order for the genes to be expressed, the plasmid DNA must be taken up by the host bacterial cell and recognized by the enzymes and proteins provided by the host for transcription and replication. A cell that is capable of taking up DNA is termed “competent.” Making cells competent usually involves a change in the ionic strength of the medium. Heating the cells in the presence of cations (e.g., calcium) causes the membrane to become permeable to DNA. A competent cell that is replicating the plasmid DNA is considered transformed. Cells lose plasmids as they grow and divide. It is imperative that the cell is grown in the presence of some selection agent and this can be an antibiotic that the cell would otherwise be susceptible to.

Replication of a plasmid containing the gene for antibiotic resistance will enable cells that hold onto the plasmid to grow in the presence of the antibiotic.

Transformation efficiency is defined as the number of colony forming units (cfu) produced by 1 µg of supercoiled plasmid DNA. Each cfu is one cell that has grown and multiplied enough times that it is visible on the agar plate as a pile of cells, or colony, to the naked eye. Each cell in that colony is identical to the original cell. Using a known amount of DNA to transform a known amount of cells, one can calculate the transformation efficiency, or how many cells actually become transformed.

One way to increase the transformation efficiency is to use SOC medium as a transformation recovery medium. Incubating the competent cells and DNA in S.O.C. medium will increase the number of transformants and, therefore, the transformation efficiency. These cells are then grown on LB plates containing a selection agent to select for transformants.

Standard Protocol for the Transformation of *E. Coli*

1. Transfer an aliquot of the DNA to be transformed (10 µL or less) into a cold sterile 1.5 mL microcentrifuge tube, and keep it on ice.
2. Thaw an aliquot of frozen competent cells on ice.
3. Gently resuspend the cells and transfer 100 µL of the cell suspension into the microcentrifuge tube with the DNA, mix gently (do not vortex), and keep on ice for 20 minutes.
4. Transfer the tube to a 42°C water bath or heating block for 90 seconds
5. Add 500 µL room temperature S.O.C. medium to the cells and incubate for 60-90 minutes at 37°C.
6. Plate out 50, 100, and 200 µL aliquots on LB agar plates containing the relevant selection antibiotic(s) to select for transformants. Incubate the plates at 37°C overnight until colonies are visible.

	Other Relevant Products	Catalog No.	Size
SOC Medium, Liquid			
Storage:	Miller's LB Broth	46-050-CM	6 x 1 L
Shipping:	Ampicillin	61-238-RH	1 x 10g
Cat. No:		61-238-RM	1 x 100g
Size:	Carbenicillin	46-100-RG	1 x 5g
Formulation:	Kanamycin Sulfate, Liquid	30-006-CF	6 x 50 mL
2% Tryptone	Kanamycin Sulfate, Powder	61-176-RG	1 x 5g
0.5% Yeast Extract	Tetracycline Hydrochloride	61-242-RG	1 x 5g
10 mM NaCl	LG Agar	BPL-2010	100 x 10/sleeve
2.5 mM KCl	LG + Chloramphenicol	BPL-2505	100 mm
10 mM MgCl ₂			
10 mM MgSO ₄			
20 mM Glucose			